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U.S. Application No.

Unassigned

09/831497

International Application. No.

International Filing Date

Priority Date Claimed

PCT/FR99/02763

November 10, 1999

November 10, 1998

Title of Invention

**USE OF PUROINDOLINE FOR PREPARING BISCUITS**

Applicants For DO/EO/US

**Laurence DUBREIL, Anne LE GUILLOU and Alette VEREL**

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventors (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 14. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☒ Other items or information:
  - a. WIPO Publication WO 00/27209 - front cover page only
  - b. PCT/IB/304
  - c. PCT/IB/308
  - d. PCT/IPEA/409

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INTERNATIONAL APPLICATION NO.

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15. [X] The following fees are submitted:

**Basic National Fee (37 CFR 1.492(a)(1)-(5)):**

Search Report has been prepared by the EPO or JPO.....\$860.00

International preliminary examination fee paid to

USPTO (37 CFR 1.482).....\$690.00

No international preliminary examination fee paid to

USPTO (37 CFR 1.482) but international search fee

paid to USPTO (37 CFR 1.445(a)(2)).....\$710.00

Neither international preliminary examination fee

(37 CFR 1.482) nor international search fee

(37 CFR 1.445(a)(2)) paid to USPTO.....\$1000.00

International preliminary examination fee paid to USPTO

(37 CFR 1.482) and all claims satisfied provisions

of PCT Article 33(2)-(4).....\$100.00

**ENTER APPROPRIATE BASIC FEE AMOUNT = \$ 860.00**

Surcharge of \$130.00 for furnishing the oath or declaration later than

[ ] 20 [ ] 30 months from the earliest claimed priority date

(37 CFR 1.492(e)).

\$

Claims	Number Filed	Number Extra	Rate	
Total Claims	16 - 20 =	0	X \$18.00	\$
Independent Claim	4 - 3 =	1	X \$80.00	\$ 80.00
Multiple dependent claim(s) (if applicable)			+ \$270.00	\$
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$

Reduction by 1/2 for filing by small entity, if applicable. Verified

Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28)

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**SUBTOTAL = \$ 940.00**

Processing fee of \$130.00 for furnishing the English translation later

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(37 CFR 1.492(f)).

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**TOTAL NATIONAL FEE = \$ 940.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The

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**TOTAL FEES ENCLOSED = \$940.00**

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- a. [ ] A check in the amount of **\$-0-** to cover the above fees is enclosed.
- b. [X] Please charge my Deposit Account No. 50-0310 in the amount of **\$940.00** to cover the remainder above fees. A duplicate copy of this sheet is enclosed.
- c. [X] **Except** for issue fees payable under 37 C.F.R. §1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 CFR §1.16 and §1.17 which may be required, or credit any overpayment to Deposit Account No. 50-0310.

Customer No. 009629

SEND ALL CORRESPONDENCE TO:

Morgan, Lewis &amp; Bockius LLP

1800 M Street, N.W.

Washington, D.C. 20036

(202) 467-7000

*Elizabeth C. Weimar*  
 Elizabeth C. Weimar  
 Reg. No. 44,478

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JC03 Rec'd PCT/PTC 10 MAY 2001

PATENT  
Attorney Docket 045636-5044

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of: **Laurence DUBREIL *et al.*** )  
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National Stage of )  
International Application: **PCT/FR99/02763** )  
 )  
International Filing Date: **November 10, 1999** )  
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Application No. **Not Assigned** ) **Group Art Unit: Not Assigned**  
 )  
Date of National Stage Entry: **May 10, 2001** )  
 ) **Examiner: Not Assigned**  
For: **USE OF PUROINDOLINE FOR** )  
**PREPARING BISCUITS** )

**PRELIMINARY AMENDMENT**

Prior to the examination of the above-identified application, please amend the application as follows:

**In the Claims:**

Please cancel claims 1 - 14 without prejudice or disclaimer.

Please add claims 15 - 30 as follows:

15. A method of controlling the texture and/or the density of a biscuit comprising combining an amount of at least one puroindoline effective for modifying the texture and/or the density of the final baked product with at least one of the other ingredients prior to baking.

16. The method of claim 15 wherein the amount of puroindoline is between 0.02 and 5% by weight relative to the weight of the flour.
17. The method of claim 15 wherein the puroindoline is combined with the flour intended for the preparation of the biscuit.
18. The method of claim 15 wherein the puroindoline is combined with the dough intended for the preparation of the biscuit.
19. The method of claim 15 wherein the amount of puroindoline is effective to increase the firmness of the biscuit.
20. The method of claim 15 wherein the amount of puroindoline is effective to control the density of a biscuit prepared from a dough with a fat content of between 2 and 30% of the total weight of the dough.
21. The method of claim 20 wherein the amount of puroindoline is effective to reduce the density of a hard biscuit prepared from a dough with a fat content of between 2 and 20% of the total weight of the dough.
22. The method of claim 20 wherein the amount of puroindoline is effective to reduce the density of a soft biscuit prepared from a dough with a fat content of between 2 and 30% of the total weight of the dough.

23. The method of claim 20 wherein the amount of puroindoline is effective to reduce the density of a puff biscuit prepared from a dough with a fat content less than or equal to 4% of the total weight of the dough.

24. The method of claim 20 wherein the amount of puroindoline is effective to increase the density of a puff biscuit prepared from a dough without added emulsifier and which dough has a fat content greater than or equal to 7% of the total weight of the dough.

25. A method of increasing the firmness of a biscuit comprising combining a flour with a puroindoline content greater than 0.2% of the dry weight of the flour with the additional biscuit ingredients.

26. The method of claim 25 wherein the puroindoline content of the flour is between 0.2 and 2% of the dry weight of the flour.

27. A biscuit comprising a flour with a puroindoline content greater than 0.2% of the dry weight of the flour.

28. The biscuit of claim 27 wherein the flour has a puroindoline content between 0.2 and 2% of the dry weight of the flour.

29. A biscuit dough comprising a flour with a puroindoline content greater than 0.2% of the dry weight of the flour.

30. The biscuit dough of claim 29 wherein the flour has a puroindoline content between 0.2 and 2% of the dry weight of the flour.

**REMARKS**

Applicants respectfully submit that no prohibited new matter has been introduced by this Preliminary Amendment and that claims 15 to 30 are drawn to the same invention as claims 1-14 of International Application PCT/FR99/02763. The changes to the claims represent changes in formalities so as to bring the claims into compliance with the rules of practice in the United States, such as: multiply dependent claims may not depend from multiply dependent claims (see original claims 5, 6 and 10), "use" claims are not a recognized category of invention (see original claims 1 to 12); and to provide established claim terminology to describe the intended scope of the claims, i.e. incorporation of the term "comprising" (see all of the original claims). These changes do not narrow the claimed subject matter presented and examined in the corresponding International Application.

Respectfully submitted,

**MORGAN, LEWIS & BOCKIUS, LLP**

By: Elizabeth C. Weimar  
Elizabeth C. Weimar  
Reg. No. 44,478

Date: **May 10, 2001**

MORGAN, LEWIS & BOCKIUS  
1800 M. Street, NW  
Washington, D.C. 20036-5869  
(202) 467-7812

4/PR+5

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WO 00/27209

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## USE OF PUROINDOLINE FOR PREPARING BISCUITS

The invention relates to the use of puroindolines as additive in biscuit manufacture.

5 Puroindolines are proteins which are present in wheat grain and which have the capacity to interact with lipids. Two isoforms of puroindolines, respectively called puroindoline a and puroindoline b, have been characterized. They are basic proteins (pI  
10 ~10) of low molecular weight (about 13 kDa); they comprise 10 cysteine residues, forming 5 disulfide bridges, and a domain rich in tryptophan residues [BLOCHET et al., in: "Gluten Proteins 1990"; (BUSHUK W. and TKACHUK R., eds), AACC, St Paul Minnesota, pp 314-  
15 325 (1991); BLOCHET et al., FEBS Lett., 329: 336-340, (1993).

Moreover, a recent publication [GIROUX and MORRIS, Proceedings of the National Academy of Sciences, vol. 95 p. 6262-6266, (1998)] indicates that  
20 the protein called friabilin, considered as a biochemical marker for wheat grain hardness [GREENWELL and SCHOFIELD, Cereal Chem., 63, 369-380 (1986)], is a heterodimer of puroindolines a and b which is involved in controlling the hardness of the endosperm.

25 Puroindolines possess specific surfactant properties linked to their high affinity for lipids; they have in particular a high foaming power which is increased in the presence of polar lipids [DUBREIL et al., J. Agric. Food Chem., 45: 108-116 (1997); WILDE et  
30 al., J. Agric. Food Chem., 41, 1570-1576 (1993)]. It has thus, for example, been shown that the addition of puroindoline made it possible to restore the foaming properties of beer supplemented with stearic acid, phospholipids or triglycerides [CLARK et al., J. Inst.  
35 Brew. 100, 23-25 (1994)], or those of egg white supplemented with oil [HUSBAND et al., in: "Food Macromolecules and Colloids" (DICKINSON E. and LODENT

D., eds), Royal Society of Chemistry, London, pp. 285-296, (1995)].

Recent work relating to the potential applications of the properties of puroindolines in the context of the manufacture of bread shows that the addition of a small quantity of puroindoline (0.1% relative to the weight of the flour) considerably modifies the rheological properties of the dough and the structure of the bread crumb [DUBREIL et al., Cereal Chem. 75, 2: 222-229, (1998)]. However, the effect of puroindolines on the volume of the final product after baking varies markedly according to the baking quality of the flour used (which is linked for a large part to its glutenin composition). When puroindolines are added to flour with good baking quality, a reduction of the order of 10% in bread volume (in other words an increase in its density) is observed compared with bread made without addition of puroindolines; this decrease in volume reaches 20% if flour of poor baking quality is used. On the other hand, if puroindolines are added to flour of average baking quality (resulting from the mixing of the above two), an increase in bread volume of the order of 20% is observed.

The inventors have undertaken the study of the effect of puroindolines on preparations which are more complex than bread dough, such as the doughs normally used in pastry and biscuit manufacture.

They have thus observed that the addition of puroindoline during the preparation of biscuits induced a significant effect on the texture and/or the density of the finished product, and that it was possible to independently control these two parameters.

The subject of the present invention is the use of puroindoline as additive which makes it possible to control the texture and/or the density of a biscuit.

For the purposes of the present invention, the term "puroindoline" is understood to mean not only the wheat puroindolines a and b mentioned above, but also



any protein or any peptide possessing functional and structural characteristics similar to those of said wheat puroindolines, and in particular any protein consisting of at least one polypeptide chain comprising  
5 at least 8 cysteine residues involved in disulfide bridges, and a tryptophan-rich domain, and possessing surfactant properties similar to those of said wheat puroindoline.

This includes in particular any natural or  
10 recombinant protein consisting of at least one polypeptide chain having the sequence of one of the allelic variants or isoforms of wheat puroindolines, or of homologous proteins present in other cereals such as oats, barley, triticale and rye, or of at least one  
15 polypeptide chain comprising at least the portions characteristic of said sequence, and which are necessary for the functional properties of puroindolines, as defined above.

These puroindolines may be used in the form of  
20 monomers as well as in the form of homomeric or heteromeric assemblies.

For the purposes of the present invention, the term "biscuit" is understood to mean any product obtained by baking a mixture, generally in the form of  
25 dough, comprising, in addition to flour obtained from cereal(s), other ingredients providing proteins, carbohydrates and/or lipids; this may include for example proteins of animal origin such as egg or milk proteins, and the like, proteins of plant origin such  
30 as soybean proteins, and the like, sugar, fatty substances of animal or plant origin. The dough is prepared from said ingredients according to any of the conventional techniques used in biscuit manufacture, which are known per se; it is thus possible, for  
35 example, to prepare a dough which is laminated, or formed by a rotary press, (so as to obtain hard biscuit-type products), a leavened dough so as to obtain cake-type products), a puff dough (so as to obtain genoese-type products), a fermented dough so as

to obtain "cracker" type products, and the like. It is also possible to prepare a biscuit by carrying out all or part of the mixing, kneading and baking of the ingredients in a cooker-extruder.

5 Earlier work in the bakery sector mentioned above has shown that the addition of puroindolines to flours free of endogenous puroindolines resulted in an increase in the density, and therefore a decrease in the volume of the bread [DUBREIL et al., publication  
10 cited above (1997)]. It could therefore be assumed that the use of these same flours, supplemented with puroindolines, in biscuit manufacture would result in similar effects.

However, the inventors have observed,  
15 surprisingly, that the addition of puroindoline in the context of the preparation of biscuits only resulted in an increase in density in the specific case of puff biscuits prepared from a dough with a high content (that is to say greater than 7%) of fat, and could, in  
20 this case, be counterbalanced by the addition of emulsifiers. In the other cases, a decrease in density is on the contrary observed; moreover, it is observed that the addition of puroindolines results in all cases in an increase in the firmness of soft biscuits and of  
25 puff biscuits.

The effects of the use of puroindoline may be modulated according to the water content of the dough and/or its fat content and/or the quantity of emulsifier which it contains.

30 The decrease in density induced by the addition of puroindolines is for example lower in the case of the products obtained from a dough containing only a small amount of water (laminated hard biscuits for example).

35 The effect on the decrease in density also becomes less important when the content of fatty substances increases; as indicated above, for a high content of fatty substances, by contrast, an increase in density is observed in the case of puff biscuits.

However, in puff biscuits containing fat and puroindolines, the decrease in density is promoted by the addition of emulsifiers such as lecithin, mono- and diglycerides of fatty acids, acetic, lactic, citric or tartaric esters of mono- and diglycerides of fatty acids, mono- and diacetyl tartaric esters of mono- and diglycerides of fatty acids, mixed acetic and tartaric esters of mono- and diglycerides of fatty acids, sucroesters of fatty acids, polyglyceric esters of fatty acids, 1,2-propanediol esters of fatty acids, sodium 2-stearoyllactylate, calcium 2-stearoyllactylate. The emulsifiers make it possible to counterbalance the effect of a high content of fatty substances which are likely to trap the puroindolines.

In accordance with the invention, said puroindoline may for example be used:

- to reduce the density of a puff biscuit obtained from a dough whose fat content is less than or equal to 4% of the total weight of the dough;
- to reduce the density of a hard or soft, non-puff dough biscuit obtained from a dough whose fat content is between 2% and 30%, preferably between 2 and 20%, in the case of a hard biscuit, of the total weight of the dough;
- to increase the firmness of a soft or puff dough biscuit; this increase in firmness contributes in particular to the obtaining of a feeling of melting in the mouth, which is appreciated by consumers.

Puroindoline induces a decrease in density greater than that observed, under the same conditions, with products normally used for reducing the density of biscuits, such as ovalbumin; it can therefore be advantageously used as a replacement for all or some of these products, or of ingredients containing them, in particular eggs. The effect on the reduction in density will be all the more marked as the reduction in the quantity of eggs causes a decrease in the total content of fatty substances in the finished product. In addition, since eggs constitute a highly variable raw

material in terms of quality, their replacement with puroindoline therefore allows, for the same density, better reproducibility of the finished product.

5 The addition of puroindoline can also make it possible to improve a flour of poor biscuit-making quality, and to obtain from it biscuits of similar density to those obtained from a flour of good biscuit-making quality.

10 For the use of the present invention, puroindoline may be added in an amount of 0.02 to 5%, preferably 0.05 to 2% of the dry weight of the flour used for the preparation of the biscuit, according to the initial puroindoline content of the flour, and the final content which it is desired to obtain.

15 It is possible to use a purified puroindoline preparation (containing a single isoform or a mixture of isoforms); it is also possible to use a cereal fraction (for example gluten or starch) enriched with puroindoline.

20 The flours previously used in biscuit manufacture have a puroindoline content which does not exceed 0.2% (of the dry weight of the flour). The invention also includes the use, for the preparation of biscuits, of flours having a puroindoline content  
25 greater than this value, and preferably of between 0.2 and 5%, advantageously between 0.2 and 2% of the dry weight of the flour.

The term "flour" is understood to mean not only wheat flour, but also flour from other cereals, such as  
30 rye, barley, triticale or oats, or a mixture of these flours.

It is also possible to use flours enriched with puroindolines by addition of purified puroindoline, or of a fraction rich in puroindoline, as indicated above;  
35 it is also possible to use flours obtained from varieties of cereals selected on the criterion of their richness in puroindoline, or of transgenic cereals overexpressing at least one of the isoforms of puroindoline.

The present invention also includes the biscuit doughs, as well as the biscuits which can be obtained from said flours.

5 The present invention will be understood more clearly with the aid of the additional description which follows, which refers to nonlimiting examples of the use of puroindoline in the preparation of various types of biscuit.

10 **EXAMPLE 1: INCORPORATION OF PUROINDOLINES INTO THE FLOUR**

A mixture of the a and b isoforms of puroindoline was extracted and purified using the method described by BLOCHET et al. (1991, publication cited above), modified by COMPOINT et al. [Large scale and rapid purification of plant lipid binding proteins by combining TRITON X114 phase partitioning and ion exchange chromatography, Conference on Plant Proteins from European crops, Nantes, France (1996)]; the preparation obtained is freeze-dried.

20 The flours enriched with puroindoline are obtained by mixing the freeze-dried product, which is designated in the examples below by the term "purified puroindolines", with the flour.

For the experiments described in the examples below, the mixtures were produced, on the one hand, from flour of the type normally used in biscuit manufacture, containing about 0.1% to 0.15% of endogenous puroindolines, and on the other hand from flour not containing endogenous puroindoline. When the quantity of flour used is less than 300 g, the mixtures are produced directly in the kneader. When the quantity of flour used is greater than 300 g, the mixture is homogenized beforehand by sieving the powders (flour + puroindoline) on an 800 µm sieve.

35 **EXAMPLE 2: EFFECT OF THE PUROINDOLINES ON A LAMINATED HARD BISCUIT**

. Incorporation of the puroindolines into the flour

a) Flour containing endogenous puroindolines

The freeze-dried puroindolines and flour containing 0.15% of endogenous puroindolines are mixed in a kneader, in the proportions indicated in table 1 below.

5

Table I

	Flour	Quantity of freeze-dried puroindolines added
Trial 0%	290 g	0 mg
Trial 0.05%	290 g	145 mg
Trial 0.1%	290 g	290 mg
Trial 0.2%	290 g	580 mg

In parallel, control mixtures are produced by replacing the puroindolines with the same quantity of ovalbumin, which is a protein conventionally used for increasing the volume (and therefore for reducing the density) of biscuits.

b) Flour containing no endogenous puroindoline

Another series of trials is carried out by adding 0.2% of purified puroindolines, or 0.2% of ovalbumin, to a flour containing no endogenous puroindoline.

For the two series of trials, the flour enriched with puroindolines or with ovalbumin is used like a conventional flour in the preparation of a laminated hard biscuit, according to the protocol indicated below.

**. Formula for the laminated hard biscuit**

The following ingredients are mixed in the proportions indicated below:

Flour	100
Icing sugar	30.00
Lard	8.00
Ammonium bicarbonate	1.00
Salt	0.63
Sodium bicarbonate	0.50
Sodium pyrophosphate	0.50
Water	26.00

The mixture is kneaded for 8 minutes (kneader temperature: 24°C, kneader speed: 30 rpm).

After resting for 30 minutes, the dough is laminated and cut into dough pieces with a hollow punch.

The dough pieces are baked in an oven at a temperature of 280°C for 7 min.

**Density of the laminated hard biscuits**

The mass, length and thickness are determined for each biscuit, and the density is calculated.

The results of trials a) are illustrated by figure 1, which represents the density of the biscuits as a function of the quantity of purified puroindolines (□) or of the quantity of ovalbumin (●) added to the flour (% by weight relative to the weight of the flour).

No significant difference is observed between the biscuits manufactured from flour containing 0.15% of endogenous puroindoline, and those manufactured from this same flour enriched with 0.05% of purified puroindolines. By contrast, a decrease in the density of the biscuits of the order of 3% is obtained when the biscuits are manufactured from flour containing 0.15% of endogenous puroindoline enriched with 0.1% or 0.2% of purified puroindolines.

The results of trials b) are illustrated by figure 2, which represents the density of the biscuits obtained from a flour containing no endogenous puroindoline, with no additive, or supplemented with 0.2% (by weight relative to the weight of the flour) of purified puroindolines, or of ovalbumin.

Legend to Figure 2:

- Flour with no additive:



- Flour + 0.2% puroindoline:



- Flour + 0.2% ovalbumin:



The results of the two series of trials show that the addition of puroindoline induces a decrease in

the density of the biscuits which is markedly greater than that induced by ovalbumin.

**EXAMPLE 3: EFFECT OF THE PUROINDOLINES ON A SOFT BISCUIT**

5           The biscuits are made according to the formula (% by weight) indicated in table II below:

Table II

FLOUR	30%
SUGAR	30%
EGGS	4.5%
FAT	10%
GLYCERIN	4%
WATER	18%
WHEY	2%
BAKING POWDERS	1.1%
SALT	0.4%
LECITHIN	0.4%

10           Three different flours are used: a flour containing no puroindoline (control 0% of puroindoline), the same flour to which 0.1% by weight of purified puroindolines relative to the weight of flour is added and a flour containing 0.1% of endogenous puroindolines (control endogenous  
15           puroindoline).

20           For the production of flour enriched with puroindoline the flour is sieved with the puroindolines using an 800  $\mu$ m sieve. This enriched flour is then sieved again with the rest of the powders immediately before use.

          These powders are respectively: baking powders, salt, lecithin, sugar, powdered eggs. They are weighed in the same container and mixed with the flour immediately before use.

25           Mixing with the liquid ingredients is then carried out and the baking is carried out for 10 min at 180°C.



The density of the biscuits obtained and the variation of their texture during storage are measured. The measurements are carried out according to the following protocols:

5 Density:

Slices 2 cm in diameter are cut out in the biscuit with a hollow punch and weighed. The density is determined according to the following formula:

$$\text{Density} = m / (w \cdot r^2 h / 10)$$

10 m = mass

r = radius

h = height of the slices.

Texture:

The texture is measured with the aid of an INSTRON penetrometer; a roll, cut out in the biscuit to be tested, is compressed at a constant rate. The force-movement of compression curve is characteristic of the sample studied. The measurement of the mean force necessary to compress the product makes it possible to determine its firmness.

The measurement parameters are the following:




- modulus of compression: roll 2.5 cm in diameter
- distance between the plates: 15 mm
- rate of traverse: 40 mm/mm
- 25 - measurement load at origin H0: 0.1 N
- minimum safe load: 100 N
- maximum (safe) movement: 13
- initial compression 0%
- final compression: 50%
- 30 - peak detection criterion: 0.1 N
- max. default value 1: 25%

Results:

Density

The results are illustrated by figure 3.

35 Legend to figure 3:

- Flour containing 0.1% of endogenous puroindoline: 
- Flour without endogenous puroindoline: 
- Flour without endogenous puroindoline:  
+ 0.1% puroindolines: 

The biscuits obtained from flour having no endogenous puroindoline are more dense than the biscuits manufactured from flour containing 0.1% of endogenous puroindoline. The addition of 0.1% of purified puroindolines to flour containing no endogenous puroindoline causes a decrease of about 6% in the density of the biscuit; the soft biscuits obtained in this manner have a density similar to that of the biscuits obtained from flour containing 0.1% of endogenous puroindoline.

#### Texture

The results are illustrated by Figure 4 which represents the variation of the mean force (Fmean) over time, for the biscuits obtained from the various flours tested: flour containing 0.1% of endogenous puroindoline ( $\blacktriangle$ ); flour without endogenous puroindoline ( $\blacklozenge$ ); flour without endogenous puroindoline supplemented with 0.1% (by weight relative to the weight of the flour) of purified puroindolines ( $\blacksquare$ ).

These results show that the addition of puroindoline has a significant effect on the texture of the biscuit. The biscuits obtained from the flour without endogenous puroindoline supplemented with purified puroindolines have a firmer texture after 29 and 56 days of storage than the biscuits obtained from flour containing 0.1% of endogenous puroindoline, or from the flour without endogenous puroindoline. From 80 days of storage (which is more than the period normally separating the manufacture of the product from its consumption), the difference in texture between the products is no longer significant.

#### EXAMPLE 4: EFFECT OF PUROINDOLINES ON A PUFF BISCUIT

The biscuits are made according to the formula indicated in table III below:

Table III

	In %	In grams
Flour	35	500
Granulated sugar	35	500
Liquid whole eggs	30	450

Two different flours are used: a flour containing no endogenous puroindoline, and the same flour enriched with 0.1% by weight of purified puroindolines relative to the weight of the flour.

The biscuit dough thus obtained contains about 4% of fatty substances provided by the eggs and the flour.


The density and the texture of the biscuits obtained are measured as indicated in example 3 above.


### Results

#### Density

The results are illustrated by Figure 5.

Legend to Figure 5:

- Flour without endogenous puroindolines: 

- Flour without endogenous puroindolines  
+ 0.1% puroindolines: 

These results show that the addition of 0.1% of purified puroindolines causes a decrease of 14% in the density of the biscuits.

#### Texture

The results are illustrated by Figure 6 which represents the variation in the mean force (Fmean) during storage, for the biscuits obtained from flour with no endogenous puroindoline (◆), or from flour with no endogenous puroindoline, supplemented with 0.1% (by weight relative to the weight of the flour) of purified puroindolines (■).

The puff biscuits containing purified puroindolines possess a firmer texture, which results in a firmness greater than 13%, at 61 days of storage.

This effect is greater than that, described in Example 3, observed in the case of soft biscuits.

**EXAMPLE 5: EFFECT OF PUROINDOLINES ON A PUFF BISCUIT CONTAINING 3% OF RAPESEED OIL**

5    . Incorporation of the puroindolines into the flour

The biscuits are made according to the formula indicated in table IV below:

Table IV

	In %	In gram
Flour	33.5	500
Rapeseed oil	3	50
Granulated sugar	33.5	500
Liquid whole eggs	30	450

10    Two different flours are used: a flour containing no endogenous puroindoline, and the same flour enriched with 0.1% of purified puroindolines.

The rapeseed oil is added before the overrun stage.


15    The biscuit dough thus obtained contains about 7% of fatty substances, taking into account those provided by the eggs and the flour.


The density and the temperature of the biscuits obtained are measured as indicated in Example 3 above.

Density

20    The results are illustrated by figure 7.

Legend to figure 7:

- Flour without endogenous puroindolines: 

- Flour without endogenous puroindolines  
+ 0.1% puroindolines: 

25    The addition of 0.1% of purified puroindolines to the flour containing no endogenous puroindoline results in a significant increase, of the order of 20%, in the density of the biscuits.

. Texture

30    The results are illustrated by figure 6 which represents the variation in the mean force (Fmean) during storage, for the biscuits obtained from flour without endogenous puroindoline (◆), or from flour

without endogenous puroindoline, supplemented with 0.1% (by weight relative to the weight of the flour) of purified puroindolines (■).

5 The biscuits obtained from the flour supplemented with puroindolines have, at 35 days of storage, a firmness 65% greater than that of the biscuits obtained from the flour without puroindoline.

10 The results obtained show that the effect of the puroindolines on the density of the puff biscuits depends on the fat content of the formula. It is thus possible to use puroindolines to decrease or on the contrary to increase the density of this type of product, according to its fat content.

15 The effect of the puroindolines on the texture also appears to be linked to the fat content; however, it is always in the same direction, that is to say toward an increase in firmness.

## CLAIMS

1. The use of puroindoline as additive which makes it possible to control the texture and/or the density of a biscuit.
2. The use as claimed in claim 1, characterized in that the puroindoline is used in an amount of 0.02 to 5% by weight relative to the weight of the flour.
3. The use as claimed in either of claims 1 and 2, characterized in that said puroindoline is added to the flour intended for the preparation of said biscuit.
4. The use as claimed in either of claims 1 and 2, characterized in that said puroindoline is added to the dough intended for the preparation of said biscuit.
5. The use as claimed in any one of claims 1 to 4, characterized in that the puroindoline is used to increase the firmness of said biscuit.
6. The use as claimed in any one of claims 1 to 5, characterized in that said puroindoline is used to control the density of a biscuit obtained from a dough whose fat content is between 2 and 30% of the total weight of the dough.
7. The use as claimed in claim 6, characterized in that said puroindoline is used to reduce the density of a hard biscuit obtained from a dough whose fat content is between 2 and 20% of the total weight of the dough.
8. The use as claimed in claim 6, characterized in that said puroindoline is used to reduce the density of a soft biscuit obtained from a dough whose fat content is between 2 and 30% of the total weight of the dough.
9. The use as claimed in claim 6, characterized in that said puroindoline is used to reduce the density of a puff biscuit obtained from a dough whose fat content is less than or equal to 4% of the total weight of the dough.
10. The use as claimed in any one of claims 1 to 5, characterized in that said puroindoline is used to increase the density of a puff biscuit obtained from a

dough without added emulsifier whose total fat content is greater than or equal to 7% of the total weight of the dough.

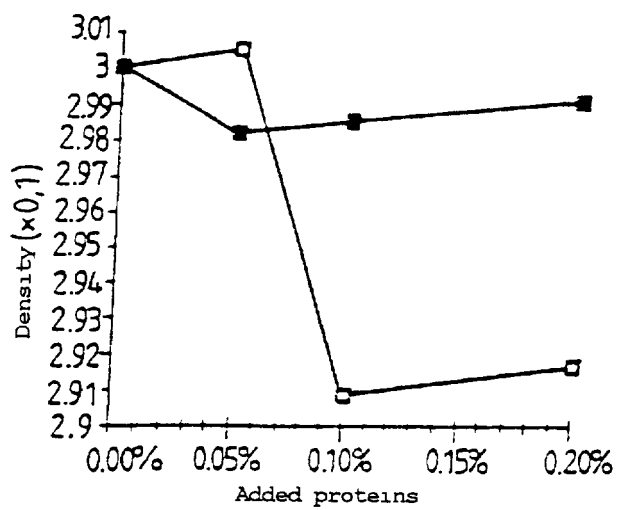
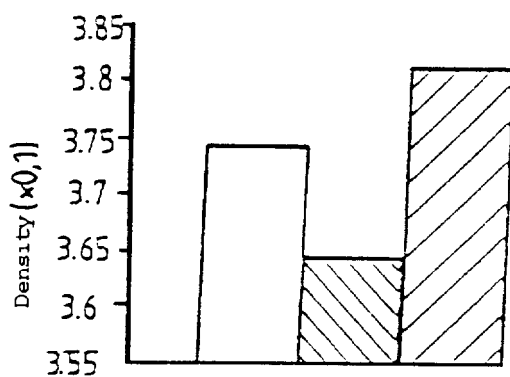
11. The use of a flour whose puroindoline content  
5 is greater than 0.2% of the dry weight of the flour, for the preparation of biscuits.

12. The use as claimed in claim 11, characterized in that the puroindoline content of said flour is between 0.2% and 2% of the dry weight of the flour.

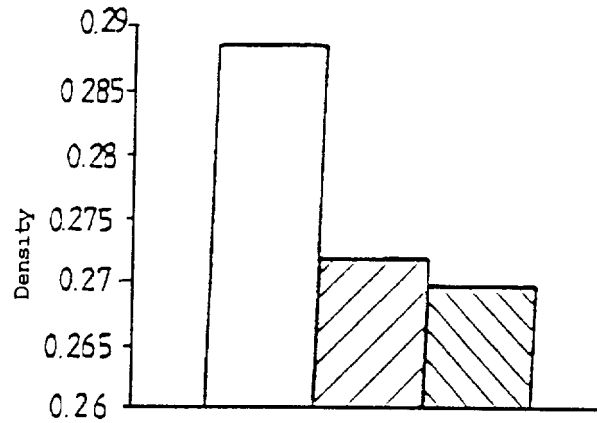
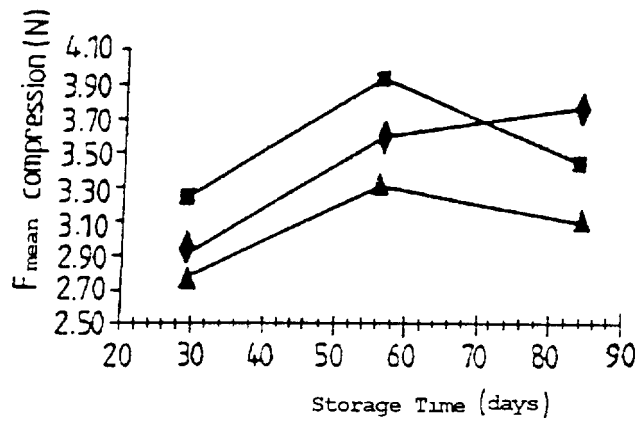
10 13. A biscuit which can be obtained from a flour as defined in either of claims 11 and 12.

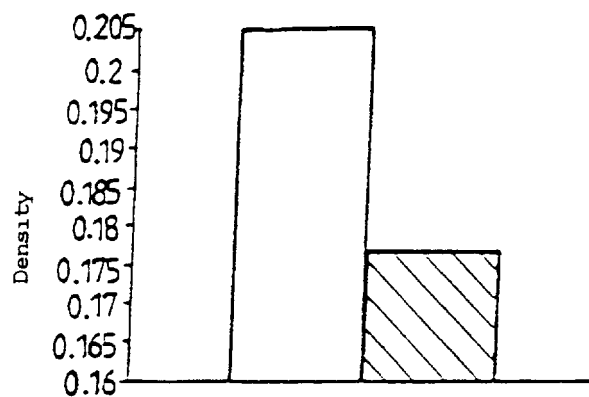
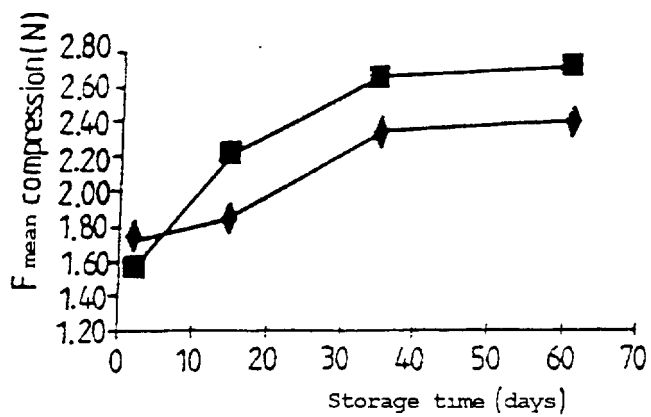
14. A biscuit dough which can be obtained from a flour as defined in either of claims 11 and 12.

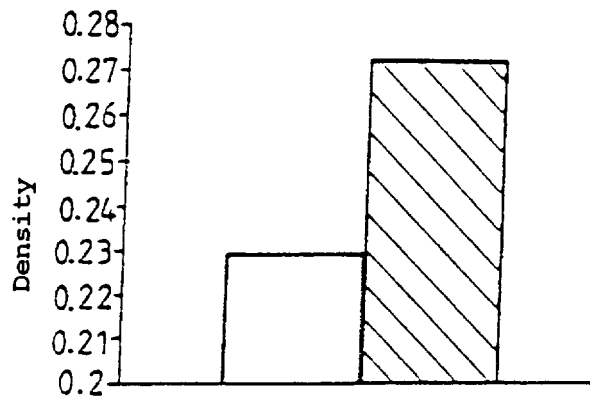
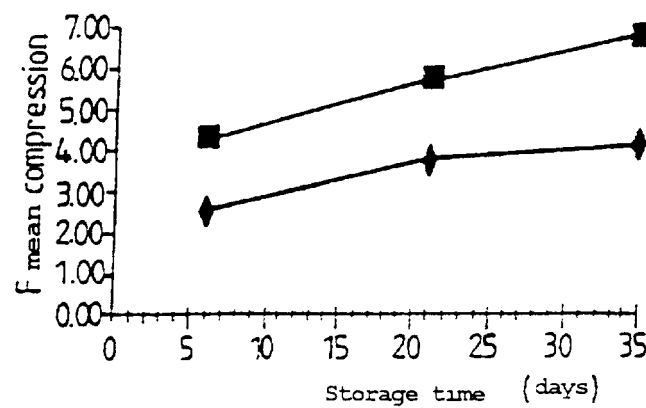
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FIG.1FIG.2



FIG.3FIG.4

FIG.5FIG.6

FIG.7FIG.8

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**U.S. DEPARTMENT OF COMMERCE  
Patent and Trademark Office

ATTORNEY DOCKET NO. :

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled :

**USE OF PUROINDOLINE FOR PREPARING BISCUITS**

the specification of which :

is attached hereto ; or

was filed as United States application Serial No. \_\_\_\_\_ on \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable) ; or

was filed as a PCT international application Number PCT/FR99/02763 on November 10, 1999 and was amended under PCT article 19 on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose the U.S. Patent and Trademark Office information which is material to the patentability of claims presented in this application in accordance with Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed :

**PRIOR FOREIGN APPLICATION(S) :**

COUNTRY (if PCT, indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
FRANCE	98/14135	10 November 1998	X

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I hereby claim the benefits under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

U.S. PROVISIONAL APPLICATIONS

U.S. PROVISIONAL APPLICATION NO.

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I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or §365 ( c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the U.S Patent and Trademark Office all information known to me to be material to the patentability of claims represented in this application in accordance with Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application :

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U.S. APPLICATIONS

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**POWER OF ATTORNEY:** As a named inventor, I hereby appoint the registered practitioners of Morgan, Lewis & Bockius LLP included in the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct all correspondence be addressed to that Customer Number.

**Customer Number : 009629**

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	<u>1-10</u> <b>DUBREIL Laurence</b>	
RESIDENCE & CITIZENSHIP	LE LOROUX BOTTEREAU, FRENCH <u>FRX</u>	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	Le Tertre 44430 LE LOROUX BOTTEREAU FRANCE	
FIRST OR SOLE INVENTOR'S SIGNATURE	<u>Dubreil</u>	DATE <u>13/06/01</u>
FULL NAME OF SECOND INVENTOR	<u>2-00</u> <b>LE GUILLOU Anne</b>	
RESIDENCE & CITIZENSHIP	FRESNES, FRENCH <u>FRX</u>	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	72, Rue Emile Zola 94260 FRESNES FRANCE	
SECOND INVENTOR'S SIGNATURE	<u>3-00</u>	DATE <u>6/6/01</u>
FULL NAME OF THIRD INVENTOR	<u>VEREL Alette</u>	
RESIDENCE & CITIZENSHIP	BIEVRES, FRENCH <u>FRX</u>	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	8, Résidence du Chat Noir 91570 BIEVRES FRANCE	
THIRD INVENTOR'S SIGNATURE	<u>Verel</u>	DATE <u>31/5/01</u>
FULL NAME OF FOURTH INVENTOR		
RESIDENCE & CITIZENSHIP		COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS		
FOURTH INVENTOR'S SIGNATURE		DATE